



UNITED STATES PATENT AND TRADEMARK OFFICE

IBERT C. WELLS

Serial No. 10/053,669

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METHODS FOR DETECTING
DEFICIENT CELLULAR MEMBRANE
TIGHTLY BOUND MAGNESIUM FOR
DISEASE DIAGNOSES

Examining Attorney:
Michael Edward Szperka

Art Group: 1644

DECLARATION UNDER RULE 1.132

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

I, Ibert C. Wells, do hereby declare and state as follows:

1. I am the same Ibert C. Wells named as Inventor on the above referenced patent application.

2. I graduated from Central Methodist College, Fayette, Missouri in 1942 with an AB in chemistry.

3. I received my PhD from St. Louis University in 1948 in the field of biochemistry, studying under Nobelist E. A. Doisy.

4. From 1948 to 1950 I held a Post-Doctoral Fellowship from the National Science Foundation under Linus Pauling, a two time Nobelist at the California Institute of Technology in Pasadena, California. Pauling is a leader in the structure of molecules. See: Linus Pauling; *The Nature of the Chemical Bond and the Structure of Molecules and Crystals*, third ed., Cornell University Press, 1960, Ithaca New York.

5. From 1950 to 1960 I was employed as an instructor and associate professor at the State University New York, Upstate Medical Center, in Syracuse, New York in the Department of Biochemistry.

6. From 1960 to 1975 I was employed by Creighton University, Omaha, Nebraska in several capacities, including Chairman of the Department of Biochemistry and Professor of Biochemistry.

7. I have worked in the field of biochemistry utilizing immuno-techniques for more than ten years.

8. Based on my experience, I am knowledgeable of the average skill of immunobiologists in the production of antibodies.

9. I am familiar with the Office Action mailed on December 1, 2005, in connection with the above-referenced patent application. In that Office Action the Examiner asserts that the specification does not teach one skilled in the art to make or use an antibody that specifically binds the amidated peptide, Phe-Gly-Leu-Met-NH₂ (SEQ ID NO:2), or a hybridoma that secretes an antibody that binds the peptide consisting of SEQ ID NO:2. In support of this assertion, the Examiner refers to Couraud, et al., J. Neurochem., 1987, 49:1708-1719. Specifically, the Examiner points to the absence of reactivity of the antibodies produced by Couraud, et al. to a tetrapeptide consisting of the non-amidated, amino acid sequence Phe-Gly-Leu-Met.

10. The Couraud et al. reference cited by Examiner discloses use of Substance P (SP) conjugated to bovine serum albumin (BSA) as the immunogen. The immunogen was prepared by reacting the carboxy terminal group of SP with the exposed amino groups on BSA in the presence of 1,5-difluoro-2,4-dinitrobenzene (See page 1709). As the consequence, the

amidated carboxy group which exists at the carboxy terminus of SP is converted to the bare carboxy group, i.e. — COOH, in place of the naturally occurring amidated carboxy group, i.e. — CONH₂. The non-amidated — COOH group can react with the amino groups of BSA, but the — CONH₂ cannot (See page 1717)

This modification of the chemical structure of SP alters the binding site at the carboxy-terminal end of SP and allows for the exposure of non-amidated segment 8-11 to the antibody producing cells. Consequently, Couraud et al. do not disclose a method that exposes an amidated SP fragment 8-11 to antibody producing cells. Furthermore, Couraud et al. report negligible cross-reactivity of their antibodies with SP (8-11) fragment, however Couraud does not report cross-reactivity to an amidated SP (8-11) fragment.

11. The monoclonal antibodies claimed in the above application are required to specifically bind SEQ ID NO:2, which is Phe-Gly-Leu-Met-NH₂. It was well known to those in the art on the priority date of the above-referenced application how to conjugate the amidated peptide of SEQ ID NO:2 to a carrier protein using a coupling protocol such that the conjugated immunogen would present the amidated carboxy group of SEQ ID NO:2 to antibody producing cells.

12 I further state that all statements made herein are true and that these statements are made with the knowledge that willful false statements and the like are punishable by fine or imprisonment or both under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the patent application and any issued patent resulting therefrom.

June 1, 2002
Dated

Ibert C. Wells
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